DISCUSSION
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The etiology of ethanol addiction is a complex interaction of psychosocial and biological factors. Ethanol freely diffuses across the blood–brain barrier and creates generalized effect all over the brain. Multiple neurotransmitter systems in various parts of brain alone as well as combined, play a prominent role in mediating the behavioural effects of ethanol that have been linked to its abuse and dependence (Koob, 1992). This undoubtedly reflects the fact that ethanol produces many pharmacological effects within the brain and body. ALDH is involved in biogenic amine metabolism (Berger & Weiner, 1977) as well as in ethanol metabolism. Brain monoamines and ALDH together plays a decisive role in ethanol addiction.

Ethanol is not stored in the body, but it is oxidized in preference over other fuels. It is reported that ethanol to a diet reduces lipid oxidation whereas oxidation of carbohydrate and protein are much less inhibited (Suter, 1992). Chronic prenatal ethanol exposure decrease cerebral cortex weights and increase locomotor activity (Abdollah et al., 1993; Catlin et al., 1993; Butters et al., 2000, Craig, 2001). Ethanol-treatment resulted in increased foetal mortality and lipid peroxidation and decreased body weight (Tanaka, 1985). Decreased food consumption was observed after ethanol intake (Macho, 2003). In the present study a decrease in body weight was observed in adult male rats during ethanol treatment. Animal studies are consistent in reporting a decrease in the body weight of rats receiving ethanol solutions as the only source of liquid (Aguiar, 2004). Different concentrations of ethanol as low as 5% (v/v), or as high as 40% (v/v) are related to decreased body weight gain (Macieira, 1997). Similar results have been reported for 20% (v/v) ethanol solution (Laure, 1990). Ethanol-
induced energy intake has no clear correlation with body weight and it is reported that ethanol energy has a low biological value (Pirola, 1976, Lands 1991; Lieber, 1991). The rate of ethanol consumption is gradually inclined upward and reached a steady state and there is no correlation observed between the rate of ethanol consumption and the body weight.

**Ethanol induced aldehyde dehydrogenase activity disparity in liver, plasma and brain regions.**

Mechanism of ethanol craving has been related to the local level of brain acetaldehyde occurring in ethanol consumption and depending on the activities of the brain and liver ethanol and acetaldehyde-metabolizing systems (Bardina, 2003). There are several reports that ethanol preference may correlate with ALDH activity more in the brain than in the liver (Amir, 1978; Socaransky et al., 1984) and this mechanism is still unknown. Oxidative deamination of monoamine neurotransmitters, catalyzed by the membrane-bound MAO generates reactive aldehyde intermediates. Aldehyde dehydrogenase, the primary enzyme responsible for acetaldehyde metabolism, is highly correlated with voluntary ethanol consumption in several strains of rats and mice (Amir, 1977). Both DOPAL and 5-HIAL are good substrates for ALDH (Ambroziak, 1991). Brain ALDH plays an important role in the biosynthesis of biogenic amines (Tipton et al., 1977). Our results showed that there is a significant increase in kinetic parameters of ALDH in cerebral cortex and it is reported that ethanol preference is related to ALDH activity in the cerebral cortex (Yamazaki, 1984). The results from ALDH enzyme analysis of brainstem showed that there is a significant decrease in the $K_m$ in brainstem without any change in $V_{max}$. There is a significant decrease in $V_{max}$ with an increase in $K_m$ in cerebellum. Disulfiram, an
ALDH inhibitor, treatment in the absence of ethanol, raises endogenous plasma and red blood cell acetaldehyde concentrations, possibly due to diminished catabolism of endogenously generated acetaldehyde (Eriksson, 1985; Rosman et al., 2000). It was observed that plasma ALDH level of ethanol treated rats increased significantly when compared to control which is suggested to be due to the increased acetaldehyde level. The results from ALDH enzyme analysis showed that there is a significant increase in the $V_{\text{max}}$ with a significant decrease in $K_m$ in the ethanol treated condition in liver when compared to control. It has been reported that colonic mucosal ALDH activities are relatively low compared to liver (Koivisto & Salaspuro, 1996). As acetaldehyde itself has many pharmacological actions (Brien & Loomis, 1983), it may act on the central nervous system (Kinoshita et al., 2001), where differences in acetaldehyde elimination may contribute to ethanol preference. Ethanol administration activates the HPA axis (Rivier & Lee, 1996). Acetaldehyde formed in brain is able to activate the HPA axis at a central level (Hiroshi et al., 2001). Difference in acetaldehyde level exerted stress on HPA axis is mediated via brainstem and plays a role in peripheral system regulation. The expression pattern of aldehyde dehydrogenase in the liver and cerebral cortex were in concordance with the enzyme activity. DA and 5-HT induced variations in the ALDH activity plays an important role in acetaldehyde metabolism.

**Brain DA and HVA changes during ethanol treatment**

Neurotransmitters can activate different subtypes of the same receptor, producing different responses in different brain cells or in different parts of the brain (Shepherd, 1994). Receptor activation causes a change in the receiving neuron. These changes may consist of a transient increase or decrease in the...
neuron's responsiveness to further messages (Grant, 1994). Due to these changes, activating mechanisms in the central nervous system prevail. The ability of ethanol to diffuse throughout the water contained in the brain and body suggested that there were probably multiple sites of ethanol action. Ethanol may produce some of its effects by interfering with signal transduction (Alling, 1993; Davis, 1996). Repeated exposure to ethanol can produce long-lasting changes in adolescent behaviour and brain function. Ethanol ingestion has been shown to induce significant change in neurotransmitter systems (Imperato, 1986; Nevo, 1995). DA and 5-HT have received special attention because of their putative role in the motivational effects of ethanol (Cloninger, 1986; Sellers, 1992; Wallis, 1993). Administration of ethanol induces DA release (Imperato, 1986; Di Chiara, 1985; O'Brien, 1995) in the caudate nucleus and nucleus accumbens of freely moving rats. DA levels in the striatum remained almost unchanged following chronic treatment with ethanol and acetaldehyde (Myers et al., 1985; Matsubara et al., 1987). Ethanol acts on the dopaminergic neurons, producing lasting changes on the system. Altered central DA function has also been implicated as influencing the propensity for ethanol consumption in humans, at least in some populations (Cowen, 1999). Changes in turnover of neurotransmitters in specific brain regions may reflect alterations in neuronal activity resulting from varied aldehyde dehydrogenase activity. This undoubtedly reflects the fact that ethanol produces many pharmacological effects within the brain. Blocking the effects of DA reduces ethanol intake by animals (Koob, 1992).

DA content decreased significantly in the cerebellum of ethanol treated rats with an increased HVA/DA turnover rate. With long-term use, adolescent rats have shown massive neuronal loss in their cerebellum and basal forebrain.
Prolonged ethanol exposure directs to neurotransmitters changes. There is a significant decrease in DA content in the cerebral cortex of ethanol treated rats with significantly increased turnover of HVA/DA. Recent studies in animals have found that as little as 2–4 days of ethanol intoxication can lead to neuronal loss in several brain areas including entorhinal cortex and hippocampal dentate gyrus (Collins, 1998). DA content was significantly decreased in the hypothalamus of ethanol treated rats. HVA/DA was significantly increased in ethanol treated rats. It indicates the alterations of the biogenic amine contents in different regions of the brain after chronic ethanol ingestion. DA content was significantly increased in brainstem of ethanol treated rats with significantly decreased HVA/DA turnover ratio in ethanol treated rats when compared to control. There is a stimulated release of biogenic amines in some brain regions and decrease in other regions due to the biphasic effect of ethanol. This has been implicated in the alterations of aldehyde dehydrogenase kinetic parameters. Vasconcelos et al., (2004) reported that duration of ethanol treatment seems to be important regarding changes in monoamine levels. Budygin et al., (2001) reported that ethanol exerts a profound effect on DA neurons, resulting in the suppression of DA neurotransmission in the striatum at high doses. DA content decreased significantly in corpus striatum of ethanol treated rats with an increased HVA/DA turnover rate. It is reported that striatal DA deficit correlated with ethanol craving (Heinz, 2005). Microdialysis experiments in rodents indicate that ethanol promotes DA release predominantly in the nucleus accumbens, a phenomenon implicated in the reinforcing effect of the drug. In humans, ethanol also promotes DA release, with a preferential effect on the ventral striatum (Boileau, 2003). It was reported (Tuomainen, 2003) that the application of ethanol to the nucleus accumbens temporarily increased DA levels.
in a dose-dependent manner. Rothblat *et al.*, (2001) demonstrated that DA and DOPAC levels were significantly decreased in the striatum of rats chronically receiving ethanol. Ethanol-induced stimulation of dopaminergic neurotransmission may encode the reinforcing properties of ethanol consumption (Heinz, 2000). Acetaldehyde increases DA neuronal activity (Marzia, 2004). The observed discrepancy in the metabolic rate of DA at different brain regions is due to ethanol induced brain alterations in the ALDH system resulting in difference in acetaldehyde elimination.

**Brain 5-HT and 5-HIAA changes during ethanol treatment**

Neurons connect with thousands of adjacent neurons. Berggren *et al.*, (2002) reported a negative correlation between prolonged and excessive ethanol consumption and central serotonergic neurotransmission due to a toxic effect of ethanol on 5-HT neurons. A significant decrease in 5-HT content was observed in the corpus striatum of ethanol treated rats and the turnover of 5-HIAA/5-HT significantly increased when compared to control. Striatal dopamine deficit is correlated with ethanol craving (Heinz, 2005). Chronic ethanol treatment decrease serotonergic neurotransmission in selective brain regions. Human studies reported damage to entorhinal cortex (Ibanez, 1995) and significant hippocampal shrinkage in ethanol addicts (Harding, 1997). It was observed a significant decrease in 5-HT content in the cerebral cortex with a significant increase in 5-HIAA/5-HT turnover rate in ethanol treated rats when compared to control. The decreased level of 5-HT observed was due to enhanced metabolic rate of 5-HT by the activated ALDH enzyme. 5-HT and its metabolic intermediates differentially regulate ethanol drinking behaviour (Wing, 1998). Ethanol has a biphasic effect on 5-HT, first raising the levels and then lowering
them (LeMarquand, 1994). Ethanol administration eventually results in depressed 5-HT levels, and thus the activity, due to increased peripheral metabolism of its precursor, l-tryptophan (Badawy, 1995). 5-HT levels remained largely the same in the nucleus accumbens following acute exposure to ethanol (Heidbreder & De, 1993). 5-HT content increased in hypothalamus with a decreased 5-HIAA/5-HT turnover rate of ethanol treated rats compared to control. Reduced density of 5-HT transporter binding in the brain might reflect reductions in the density of 5-HT terminals that might contribute to reduced central 5-HT function (Tiihonen, 1997; Chen, 1991). Chronic ethanol administration altered the serotonergic system in a time dependent manner (Uzbek et al., 1998). 5-HT content was significantly decreased in brainstem of ethanol treated rats when compared to control. Turnover rate of 5-HIAA/5-HT significantly increased in ethanol treated rats when compared to control. These results indicate alterations of the biogenic amine contents in brain regions after chronic ethanol ingestion. Stimulated release of biogenic amines in some brain regions and decreased in other regions is due to the biphasic effect of ethanol and has been implicated in the regulation of aldehyde dehydrogenase kinetic parameters. Decrease in serotonergic activity might be involved in the early phase of ethanol withdrawal (Syvalahti et al., 1988). The alterations of brain 5-HT function in the brainstem, hypothalamus, corpus striatum, cerebral cortex play an important role in the sympathetic control of ALDH enzyme regulation in liver. McBride (1995) has reported that levels of brain 5-HT is lower in ethanol-preferring rats than in non-preferring ones. 5-HT and its metabolite 5-HIAA changes at different brain regions are due to ethanol induced brain alterations in ALDH system resulting in the difference in acetaldehyde elimination.
Liver DA, 5-HT and their metabolite changes during ethanol treatment.

Aldehydes in the metabolic pathways of ethanol, DA and 5-HT are substrates for ALDH. Acetaldehyde is the initial metabolite of ethanol, which is produced in the liver following ethanol administration. Aldehyde dehydrogenase oxidizes a broad class of aldehydes to their carboxylic acids (Lindahl, 1992), involved in biogenic amine metabolism (Berger and Weiner, 1977). Ethanol intake significantly changes the liver cytosolic redox potential by increasing the NADH/NAD⁺ ratio (Smith, 1959). Although the ethanol feeding did not influence the stomach ADH and ALDH activity levels, these enzymes in the liver were affected (Wei, 1988). Decreased DA and 5-HT content in liver with an increased HVA/DA and 5-HIAA/5-HT turnover rate observed in ethanol treated rats compared to control. Over activity has been supposed to contribute to the morphological and functional degeneration of rat peripheral sympathetic nervous system. It has been observed that in patients in the preliminary stage of addiction show only functional disturbances in the liver: the increase of ethanol dehydrogenase activity with evidences for the induction of its synthesis (Kharchenko, 2001). Most of the acetaldehyde produced from ethanol is metabolized quickly to acetate by liver ALDH and hence acetaldehyde concentration in blood following ethanol administration is very low (Eriksson, 1973; Eckardt et al., 1998). Our results suggest that decreased DA and 5-HT level and increased turnover rate of metabolites may be due to the ethanol induced neurotransmitter mediated changes on aldehyde dehydrogenase.
Determining the specific neurotransmitters and receptor subtypes that may be involved in the development of the effects of ethanol addiction is the first step in developing medications to treat ethanol addiction (Hunt, 1993; Deitrich, 1996). Neuronal DA receptors are widely distributed in the central and the peripheral nervous systems at different levels. DA D2 receptor-selective agonist, quinpirole, increases renal sympathetic firing (Szabo, 1992). Compared to normal rats, the alcohol-preferring rats have a reduced supply of DA in the nucleus accumbens and a lower density of DA D2 receptors in certain areas of the limbic system (Russell et al., 1988; McBride et al. 1990; McBride et al. 1993). From our analysis we observed a decreased receptor activity in cerebral cortex, brainstem and corpus striatum in ethanol treated rats with an increased affinity. This is a mechanism to compensate the decreased DA D2 status. The brain reduces the number of DA binding sites on neurons to protect itself from a persistent oversupply of the neurotransmitter. Jan et al., (1994) suggests that severely ethanol-dependent subjects with reduced DA D2 receptor function. It is reported that striatal DA D2 receptor density is decreased in ethanol-dependent patients (Tiihonen, 1997; Volkow, 1996). Serotonergic neurotransmitter pathways have all been shown to interact at various points along the mesolimbic dopaminergic pathway to modulate its activity (Denise & Sellers, 2001). Increased YM-09151-2 binding to DA D2 receptor was observed in cerebellum and hypothalamus of ethanol treated rats compared to control. Increased density of DA D2 receptors may be a predictor of vulnerability to relapse in ethanol-dependent patients (Guardia, 2000). Repeated deprivations increase binding sites of DA D1 and DA D2 receptors in specific regions of the extended amygdala (Sari et al., 2006). The functional alterations in the DA D2 receptor kinetics in
different brain regions is due to ethanol induced central neurotransmitter system changes occurring during ethanol treatment.

**5-HT$_2$A receptor alteration in brain regions**

Change in receptor function results from direct action of ethanol on the receptor protein or molecules closely associated with the receptor in the cell membrane (Lovinger, 1993, 1994). Ethanol exposure inhibits the function of a neurotransmitter receptor; the cells may attempt to compensate for continuous inhibition by increasing the receptor numbers or by altering the molecular makeup of receptors or cell membranes so that ethanol no longer inhibits receptor function. The 5-HT$_2$ receptor appears to undergo such adaptive changes (Pandey, 1995). 5-HT$_2A$ receptor kinetics showed a functional decrease in cerebral cortex, cerebellum and liver of ethanol treated rats compared to control. There are lowered levels of 5-HT$_2A$ binding sites in the cingulate cortex, the frontal cortex and in the agranular insular cortex (Fedeli, 2002) in 7 days of high doses of ethanol treated rats. It was suggested that this decrease in 5-HT$_2A$ receptor density represented a down regulation of the receptors due to an activation of serotonergic transmission in these regions. Ethanol reduces the normal formation and growth of 5-HT neurons in the midbrain. Furthermore, the projection of 5-HT fibers, in density as well as in distribution, is reduced in the major trajectory bundle. This may affect the amount of 5-HT fibers available to the forebrain (Youssef, 2001). 5-HT$_2A$ receptor kinetics showed a functional increase in corpus striatum, hypothalamus and brainstem. Altered regulation of brain serotonergic mechanisms; changes in 5-HT$_2A$ receptor density and functioning have been observed in ethanol abuse. Dense projections from the subgenual cingulate cortex to the dorsal raphe (Freedman et al., 2000) raises the tantalizing
possibility that the subgenual cortex plays some role in regulating overall serotoninergic activity (Ursula, 2004). Altered 5-HT function in fronto-cortical areas could be linked to the genetic predisposition to high voluntary ethanol intake (Ciccocioppo et al., 1999). Preuss et al., (2001) reported an association of 5-HT2A promoter polymorphism and impulsive behaviour in ethanol dependents. The serotoninergic neurons that innervate neuroendocrine control regions in the hypothalamic paraventricular nucleus send collaterals to other limbic brain regions, notably the amygdala (Petrov et al., 1994). Hence the alterations of the serotoninergic system mediated changes during ethanol treatment calls for special attention.

**Hepatic 5-HT2A receptor alterations**

Brain plays an important regulatory role in hepatic function (Lautt, 1983). The relationship between the functional status of the liver and that of the brain has been known for centuries (Frerichs, 1860). The liver is richly innervated (Rogers & Hermann, 1983). 5-HT facilitates central sympathetic nerve activity (Kuhn et al., 1980). Autonomic nervous system has an important role in the process of hepatic cell proliferation (Tanaka et al., 1987). The role of 5-HT in regulating cortisol secretion has long been recognized (Dinan, 1996), and evidence suggests that cortisol secretion is regulated by central 5-HT2A/2C receptors (Rittenhouse, 1994). During acute stress, the HPA axis - modulate the brain's response to stress - is activated, increasing the adrenocorticotropic hormone (ACTH), which in turn increases cortisol, clearly indicating the interaction between serotonergic system and HPA axis. During ethanol intoxication and ethanol withdrawal, ACTH and cortisol are also increased. In hepatic encephalopathy and other liver diseases, neurotransmission in the brain is
reported to be altered (Basile et al., 1991; Jones, 1995; Butterworth, 1995). 5-HT$_{1A}$ agonists act centrally inhibiting sympathetic nerve discharge (McCall et al., 1987). Hypothalamic and autonomic nervous regulation of carbohydrate and amino acid metabolism was observed in the liver (Shimazu, 1981). Brainstem has direct connection with liver through the vagus nerve (Tanaka et al., 1987) and plays a regulatory role in liver function. Ethanol induced serotonergic activity alterations over ALDH enzyme leads to the increased activity of ALDH enzyme. The 5-HT system itself is altered and the number of receptor binding sites in liver is greatly reduced with an increase in affinity as a compensatory mechanism. Decrease in 5-HT$_{2A}$ receptor protein level with increased affinity is observed in our model which clearly establishes its unambiguous role in ethanol mediated receptor changes and its regulatory aspects during ethanol treatment.

**Ethanol induced ALDH, DA D$_2$ and 5-HT$_{2A}$ receptor gene expression changes**

Ethanol exposure affects multiple genes and various receptor-associated signalling pathways which regulate the expression of a multitude of downstream genes (Fan et al., 2004). The human DA D$_2$ receptor gene is an important candidate gene for ethanol addiction and/or for the modification of its severity (Blum et al., 1995; Noble, 2000; Finckh, 2001; Lu et al., 2001). Neuroadaptive changes in DA D$_2$ receptor levels occur following alcohol drinking and withdrawal. The Real-Time PCR analysis of DA D$_2$ in the hypothalamus and cerebellum of ethanol treated rats showed an increased expression in mRNA
synthesis compared to control rats. There is evidence that over expression of DA D2 attenuates alcohol drinking (Thanos et al., 2004). A modification of gene expression is the crucial component of risk that predisposes an individual towards ethanol addiction. The Real-Time PCR analysis of DA D2 in the cerebral cortex and corpus striatum of ethanol treated rats showed a decreased expression in mRNA synthesis compared to control rats. The DA D2 receptor genes are interacting with ALDH genes, there is association between the DA D2 receptor gene and alcohol dependence. Also ALDH genes are involved in dopamine metabolism (Huang et al., 2004). The Real-Time PCR analysis of ALDH in the liver and cerebral cortex of ethanol treated rats showed an increased expression in mRNA synthesis compared to control rats. Exposure to ethanol changes the patterns of gene expression in such a manner that drinking session continued and ultimately, addiction. The Real-Time PCR analysis of 5-HT2A in the liver, cerebral cortex and cerebellum of ethanol treated rats showed a decreased expression in mRNA synthesis compared to control rats. The diverged pattern of gene expression that portrays the perturbed nervous system assumes a new set point in the face of constant exposure to alcohol. The Real-Time PCR analysis of 5-HT2A in the hypothalamus, corpus striatum of ethanol treated rats showed an increased expression in mRNA synthesis compared to control rats. The differential expression DA D2 and 5-HT2A receptor genes suggests the involvement of the dopaminergic and serotonergic receptor subtype alterations during ethanol treatment in conferring functional regulation on ALDH activity.

Central, Peripheral DA, 5-HT and Liver ALDH activity

Levels of ethanol consumption are correlated with brain and liver aldehyde-oxidizing capacity (Amir, 1978; Socaransky, 1984). Alteration in the
ethanol metabolizing enzymes, specifically those enzymes responsible for the metabolism of ethanol's primary metabolite acetaldehyde, is the critical factor in the predisposition towards ethanol addiction (Haranda *et al.*, 1983; Mizoi *et al.*, 1983). The high ethanol preferring rats showed significantly lower DA and 5-HT release in the striatum and nucleus accumbens than low alcohol preferring rats (Minori, 2002). It is reported that ALDH is involved in biogenic amine metabolism (Berger & Weiner, 1977). Endogenous DA plays role in modulating norepinephrine release by human sympathetic nerves *in vivo* (Massimo, 1999). ALDH plays this role by regulating the levels of acetaldehyde in brain (Karen, 1987) and liver. Dopamine and serotonin content decreased in brain regions - cerebral cortex and corpus striatum of ethanol treated rats with an increased HVA/DA, 5-HIAA/5-HT turnover rate. Most ethanol elimination occurs by ADH and ALDH systems via oxidation of ethanol to acetaldehyde and acetic acid (Crabb, 1995). It has been observed that ethanol preferences in rats vary with the levels of brain ALDH activity (Amir, 1977; Amit *et al.*, 1980). Dopamine content increased in brainstem with an increased HVA/DA turnover rate and serotonin content decreased with an increased 5-HIAA/5-HT turnover rate in ethanol treated rats compared to control. Brain ALDH activity was significantly higher in rats preferring ethanol than in rats not preferring ethanol. With respect to implications for a biological regulator of ethanol intake, the most exciting aspect of cerebral ALDH is its apparent noninducible character in response to ethanol or acetaldehyde exposure (Socaransky *et al.*, 1984). Although the precise mechanism by which ALDH regulates voluntary ethanol intake is yet to be elucidated, these studies support the decrease in DA synthesis. The enhanced clearance of synaptic DA may cause DA hypofunction during ethanol dependence (Rothblat *et al.*, 2001) which will eventually affect the ALDH
kinetic function. Serotonin content increased in hypothalamus with a decreased 5-HIAA/5-HT turnover rate and dopamine content decreased with an increased HVA/DA turnover rate of ethanol treated rats compared to control. A significant decrease in 5-HT and DA content was observed in the liver with significantly increased turnover rate of 5-HIAA/5-HT and HVA/DA in ethanol treated rats when compared to control. These results suggest that sympathetic nerves directly involve in ethanol metabolism in the rat liver. Augmented kinetic rate of ALDH is suggested to be due to the differential regulation of DA and 5-HT system through sympathetic stimulation and peripheral control at the hepatic level. Thus, brain and liver 5-HT and their metabolic rate, 5-HT\textsubscript{2A} receptor affinity shift differentially regulates ALDH function during ethanol addiction. Monoamine neurotransmitter system alterations induce the activation of ALDH in the liver oxidation of acetaldehydes.

Dopaminergic and serotonergic regulation on kinetic parameters of aldehyde dehydrogenase

The perfusion model technique could help in identifying neurotransmitters acting as messengers in signal transfer and it is vital to identify those contributing to ALDH regulation. Lower activity of ALDH, is believed to play a preventive role against ethanol (EtOH) addiction (Goedde, 1982). Tae et al., (2006) reported a time dependent decrease in plasma acetaldehyde concentration without changing plasma ethanol concentrations observed when rats are treated with Rosiglitazone - peroxisome proliferator-activated receptor (PPAR)-\textgamma agonist - mediated by receptor-dependent activation of the PPAR-\textgamma-
retinoid X receptor (RXR) complex. Thus, the expression of aldehyde dehydrogenase could potentially be regulated by rosiglitazone by acting on PPAR response elements (PPREs) in ALDH promoter site. Administration of substances that increase the supply of 5-HT at the synapse or that directly stimulate DA D2 receptors reduce craving for ethanol (McBride et al. 1993). DA D2 receptor agonists reduce the intake of ethanol among rats that prefer ethanol, whereas DA D2 receptor antagonist increases the drinking of ethanol in these inbred animals (Dyr et al., 1993). Selective serotonin reuptake inhibitors (SSRIs) have been reported to reduce drinking in animals and also in some heavy drinking individuals (Liskow & Goodwin, 1987). Ethanol metabolism is impaired by a nonfunctional form of the enzyme aldehyde dehydrogenase (Wall & Ehlers, 1995). More than 80% of ethanol taken into the isolated rat liver recovered as free acetate in the perfusate (Yamashita, 2001). Sympathetic-nerve stimulation stimulates glycogenolysis in perfused liver (Iwai & Jungermann, 1989). The DA induced decrease in liver ALDH enzyme level represents an activation of the whole DA receptor-signalling cascade in the liver and the functional changes of 5-HT mediated affinity shift in ALDH during EtOH perfusion clearly shows the involvement of serotonergic and dopaminergic system in ALDH regulation.

**Ethanol mediated electrophysiological changes**

Ethanol interferes with synaptic firing. Central effect of ethanol is mainly based on their effect on GABAergic, glutamatergic and serotonergic transmission (Pietrzak, 2005). A characteristic feature of the EEG recording after ethanol
administration is a deceleration of the rhythms obtained from the cortex and an increase in the amplitude (Klemm & Stevens, 1974; Perrin et al., 1974). Alpha rhythm is more significant and it can be recorded in different parts of the brain. Human study suggested that ethanol decreases alpha rhythm frequency and increases its amplitude (Klemm et al., 1976; Noldy & Carlen, 1990). Acetaldehyde produces electrophysiological actions on VTA neurons in vivo, similar to those produced by ethanol, and significantly participate in ethanol-induced increment in DA neuronal activity (Marzia et al., 2004). EEG studies in the frontal region showed a prominent brain activity difference in the ethanol treated rats. Ciccocioppo et al., (1999) reported that altered 5-HT function in fronto-cortical areas could be linked to high voluntary ethanol intake. The EEG findings suggested that ethanol induced changes made rats physiologically more sensitive than control rats. Ethanol interferes with synaptic firing. Acetaldehyde also have role in electrophysiological changes. Discrepancy in the acetaldehyde metabolism is suggested to differentially stimulate electrophysiological indices. Increased cortical P1 amplitude and altered cortical EEG activity may be the neurophysiological 'risk factors' associated with high ethanol consumption in mice (Slawecki et al., 2003). It is reported that reduced central 5-HT function causes poor impulse control in ethanol addicts (Sander et al., 1995; Nielsen et al., 1994). Kahkonen et al., (2003) reported that ethanol-induced differences were most pronounced at anterior electrodes. The prefrontal cortex has been linked to impulse control because damage to this region of the brain can lead to loss of inhibitions, which is prominent in ethanol addicts. The hyper activity at the frontal cortical region observed during the EEG analysis supports the central effects of ethanol especially at the frontal region.
Thus the results suggests that DA and 5-HT through their DA D$_2$ and 5-HT$_{2A}$ receptor subtypes functionally regulate the ALDH activity in the brain regions and liver tissue of ethanol treated rats. Real-Time PCR studies confirm the DA D$_2$ & 5-HT$_{2A}$ receptor binding parameters. Perfusion studies data show that dopamine, serotonin and glucose can regulate the ALDH activity in the liver of rats. EEG studies in the frontal region showed a prominent brain activity difference in the ethanol treated rats. DA and 5-HT functional regulation of ALDH has immense clinical significant in the management of ethanol addiction.